Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

Phytohemaglutinin (PHA) Immune Challenge protocol

Prepared by Joe Casto 2001; updated by Nicole Gerlach 2006

Phytohemaglutinin (PHA) is a lectin from *Phaseolus vulgaris* (red kidney bean). An injection of PHA in solution is a standard way to measure cell-mediated immune response.

To make PHA Solution:

- 5 mg phytohemaaglutinin PHA-P (Sigma-Aldrich, Inc., L8754) dissolved in 1 mL phosphate-buffered saline. Final concentration is 0.25 mg/ 50 μL PBS. PHA is temperature-sensitive; keep solution refrigerated.

For Nestlings:

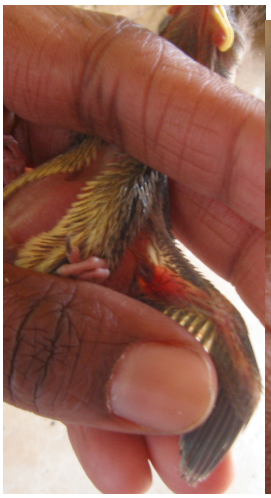
On the afternoon of day 6:

1. Weigh/measure/band nestlings as usual. If a blood sample is to be taken, do so before beginning the PHA protocol
2. Take an initial measurement of wing-web thickness (to the nearest 0.01 mm) using a pressure-sensitive digital thickness gauge (Mitutoyo No. 547-500) on the left wing.
3. Using a disposable 0.1 mL syringe and a 26 ½ gauge needle, inject 0.25 mg of PHA in 50 μL of phosphate-buffered saline (PBS) subcutaneously to the wing web.
4. Use a clean needle for each bird, or wipe the needle tip with ethanol between injections.

On the afternoon of day 7:

5. On day 7 (24 hours later), re-measure the thickness of the wing-web, and measure/weigh the nestlings again.
6. The cell-mediated immune response is the post-challenge thickness minus the pre-challenge thickness.

For adults: Follow a similar protocol as for nestlings, with a 24-hour period between injection and post-challenge measurement.




Photos by Nicole Gerlach